A Study on the Milk Coagulating Property of the Kesinai Plant (*Streblus asper*)

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Introduction

The kesinai plant (*Streblus asper*) is quite common in the northern states of peninsular Malaysia where it is grown as a windbreaker and to provide shade as well as animal feed. The sap of this plant has the property of coagulating milk and has been used by the local people to produce a yoghurt-like dairy product (non-fermented) called dadih using fresh cow or buffalo milk.

The basis for this property has not been elucidated, although it can be attributed to the presence of a specific protease. Such an enzyme would be useful industrially as a rennin-substitute for many applications in dairy technology, specifically in the manufacture of cheese. Present method of cheese production relies on rennet enzyme obtained from the stomach of calves and supply is limited. Hence this project was aimed at studying the basis for the milk coagulating property of the kesinai plant.

Materials and Methods

The kesinai plant was obtained from the farming region around Alor Setar, Kedah and grown in the Ladang of UPM to provide for the experimental material. The plant leaves were blended in a buffer to product a dark brown to black "juice". This dark coloration is due to the formation of polyphenols. Using this crude preparation to prepare dadih resulted in an unpleasant taste, hence work was done to remove the color. A protease enzyme was purified from this crude preparation using standard protein chromatographic technique, FPLC and IEF. The milk coagulating properties of the kesinai protease was compared to those of commercial milk coagulating enzymes such as Fromase and calf rennet. The milk coagulum produced was studied for its physico-chemical properties using a SEM, TEM, protein electrophoresis and texture analyzer.

Results and Discussion

A chemical method to clarify the plant extract was developed, resulting in a clear kesinai extract. This method has the potential of being patented as it can be applied to other process involving plant extracts, which produce coloration (browning) such as the fruit juice industry. The kesinai protease was purified to homogeneity and has the properties of being thermostable (active up to 80 °C), MW 31 kDa, pH optimum of 7.2 and pI of 5.2. A zymogram analysis showed that the proteolysis activity and milk coagulating activity were correlated. A comparative study of the proteolytic activity of the kesinai enzyme against Fromase and calf rennet showed that the kesinai protease has more non-specific hydrolytic action, resulting in smaller protein bands as observed in SDS-page. Thus under prolonged digestion time, more milk proteins are hydrolyzed by the enzyme. SEM and TEM analysis of the milk coagulum showed a fairly dense fibril-like structure compared to the more open 3-D network obtained using Fromase and calf-rennet.

Conclusions

The milk coagulating property of the kesinai plant is found to be due to the presence of a protease. The enzyme as purified and characterized. This enzyme has the potential to be used as a rennin-substitute in dairy technology, as well as a general protease in food processing, detergents and leather making. A chemical method for producing clear plant extract was developed with potential to be used in clarifying plant juice extract. The milk coagulum produced using kesinai protease is similar to that obtained using commercial milk-coagulating enzymes, except that under prolonged exposure to the enzyme, more milk proteins can be hydrolyzed.

Benefits from the study

The study revealed new knowledge on a plant protease with potential used in dairy technology and the food industry. This enzyme is thermostable and can be cloned for production in a biotechnology plant. A method to decolorize plant extract and plant juice was developed. Many undergraduates and postgraduates have benefited from working in this project, and a reasonably well-equipped laboratory dedicated to biochemistry and molecular biology work has been established. The study conducted also helped in establishing academic ties and research collaboration with local and overseas universities.

Literature cited in the text

None.

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Graduate Research

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